An E-learning Application on Cell and Tissue Eletroporation

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Abstract. Electroporation is an electrical increase in cell membrane permeability by means of local delivery of short and sufficiently intense voltage pulses to the target cells or tissues for biomedical and biotechnological purposes. Electroporation is used as an effective technique for delivery of variety of therapeutic agents such as chemotherapeutic drugs, DNA or other molecules, which in normal conditions do not cross cell membrane, into many different cells either in vitro or in vivo. Electroporation is used in clinical electrochemotherapy of cutaneous and subcutaneous tumors, in non-viral gene electrotransfer for gene therapy and DNA vaccination purposes, in transdermal drug delivery and new medical applications are emerging at an increasing rate. In this paper we present a web-based e-learning application which was developed in order to collect, organize and provide the knowledge and experience about cell and tissue electroporation as well as about its medical applications. The e-learning application is based on HTML, JavaScript, ASP and Macromedia Flash technologies and integrated into an interactive e-learning environment (ECHO) developed at our institution. The E-CHO enables authentication of users, statistical analysis, network traffic measurement, support for video streaming, as well as the use of various types of communications among users, such as forums, e-mail correspondence and videoconferencing.

Introduction

Electroporation is a phenomenon of cell membrane permeability increase due to local delivery of short and sufficiently intense voltage pulses via appropriate electrodes to the target cells and tissues [1]. Electroporation is used as an effective method for introduction of either small molecules (i.e. therapeutic agents such as chemotherapeutic drugs) or macromolecules (such as DNA) or other molecules which in normal conditions do not cross cell membrane. This method can be applied to many different types of human, animal or plant cells and tissues for different biomedical or biotechnological applications. In medicine this method is used in clinical electrochemotherapy of cutaneous and subcutaneous tumors and in non-viral gene electrotransfer for gene therapy and new medical applications are emerging at an increasing rate [2].

The effectiveness of cell and tissue electroporation depends on one hand on the parameters of the applied pulses such as amplitude, duration, number and repetition frequency and type of electrodes used and on the other hand on the characteristics of the cell and tissues to be electroporated. Depending on the electric pulse parameters used the electroporation can be reversible or irreversible. Namely, when the electric pulses are applied a local electric field (E) is established within the treated cells or tissues. Since the electroporation is a threshold phenomenon magnitude of local electric field need to achieve the critical reversible threshold value (E_{rev}) in order to cause structural changes in cell membrane and to trigger the increase in its permeability. The phenomenon is reversible until the magnitude of local electric field reaches the irreversible threshold value E_{irrev}, which causes permanent damages of the cell membrane. The reversible electroporation regime has to be assured in all applications in which the viability of cells have has to be preserved, such as for example electrochemotherapy or gene therapy [1]. On the other hand, in some medical and biotechnological applications such as irreversible tumor tissue ablation, liquid food sterilization of water treatment, the irreversible electroporation is used as a method for efficient cell killing. The electroporation pulse parameters can also be designed so as to trigger electrofusion of cells with electroporated membranes in fusogenic state. [2]

The key role in electroporation effectiveness plays the local electric field distribution, which can be directly modified by electric pulses and electrodes [3]. Thus, for controlled use of the method in each particular electroporation mediated application the pulse parameters and electrodes need to be specifically optimized. Realistic mathematical models validated on corresponding experimental observations are valuable tool in designing the cell and tissue electroporation level/regime. Numerical

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calculations of local electric field distribution in realistic mathematical models allow for optimization of pulse parameters and electrode geometry and their positioning. In electroporation mediated applications planning a multidisciplinary expertise is required. Namely, the collaboration and knowledge and experience exchange among the experts in the fields of medicine, biology and engineering is needed. The efficacy of electrochemotherapy can be improved with a good knowledge of parameters of the local electric field, being crucial for successful tissue electropermeabilisation and subsequently for the best electrochemotherapy treatment outcome. In electrochemotherapy a close collaboration between oncologists and electrical engineers is of great importance. To make the therapy as efficient as possible it is of great importance to transfer that knowledge to the practicing clinicians who plan or perform the treatment.

To collect, organize and transfer the acquired knowledge web-based technologies are being an indispensable tool in modern teaching. The web-based e-learning programs offer more educationally effective and enjoyable learning and teaching methods compared to the conventional learning methods such as learning through listening to spoken words. Furthermore, the use of web-based e-learning techniques enables the simulation of the users' participation in "hands-on" learning activities, which is proven to be the most retentive learning method [4].

In this paper we present a web-based e-learning application which was developed in order to collect, organize and provide the knowledge and experience about cell and tissue electroporation. The educational content is based on previously published studies from a single cell level and simplified tissue models to complex biological tissues. In the first part of the e-learning application we explain basic mechanisms underlying electroporation process. Based on simple graphical illustrations we demonstrated the influence of each of the pulse parameters, such as pulse amplitude, pulse number and duration, on electroporation of cells with different sizes, shapes and orientations with respect to the applied electric field. By using 3D animation we visualized the aqueous pore formation in cell membrane, which is most widely accepted model, among different theoretical models that describe cell membrane electroporation [2].

One of the main objectives of the presented e-learning application is to demonstrate the importance of local electric field distribution for effective electroporation of treated cells and tissues. For this purpose we used combination of numerical calculations by means of mathematical modeling and simple graphical illustrations. We demonstrated how the pulse amplitude, electrode shape and electrode positioning influence on the local electric field distribution within the treated cells and tissues. We also illustrated how the electric properties of a treated sample can modify the local electric field distribution.

We further provide main medical applications of reversible electroporation with special emphasis on electrochamotherapy. We also provide a list of applications of irreversible electroporation in medicine and biotechnology. Electrochemotherapy treatment outcome is directly related to the local electric field distribution within the target tumor tissue and its surrounding tissues [3]. Therefore, this part of e-learning application was developed in order to provide an educational material about the parameters of local electric field being crucial to make the tumor treatment as efficient as possible. The main conclusions on influential parameters on local electric field distribution can be applied also for other electroporation based applications.

The e-learning application is concluded by a test on the presented educational material. The textual and graphical information was published using HTML and integrated into an interactive elearning environment (E-CHO) [5].

1 Methodology

The e-learning web application is based on HTML, JavaScript, ASP and Macromedia Flash web technologies.

For graphical illustrations and 3-dimensional visualizations of the electroporation process on the levels of cell membrane, cell and tissues a software package 3D Studio Max was used. Based on the numerical calculations of electric filed distribution carried out with software packages COMSOL Multiphisycs and Matlab, more simple 2dimensional and 3-dimensional illustrations using software packages 3D Studio Max, Macromedia Flash, PhotoShop and CorelDraw are designed. The educational content (textual and graphical information) is published using Hypertext Markup Language (HTML). The designed e-learning application is integrated into E-CHO elearning system developed at the Faculty of electrical engineering (University of Ljubljana) by the Laboratory of telecommunications [5]. The E-CHO e-learning system is an interactive e-learning

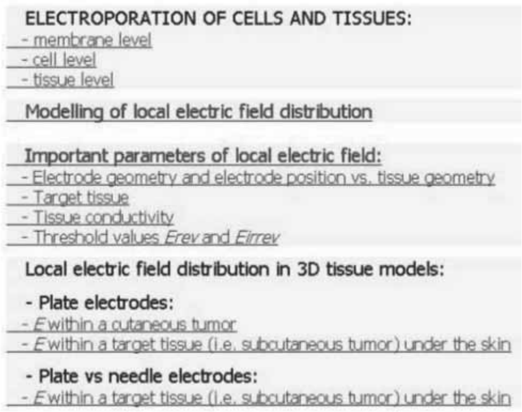

Figure 1. The structure of the e-learning application on cell and tissue electroporation

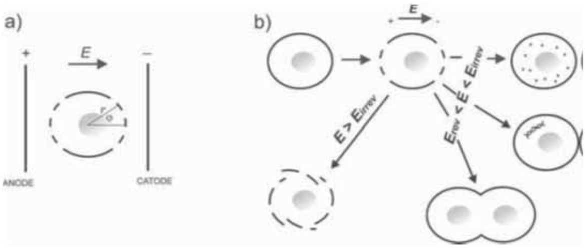

Figure 2. (a) The electroporation of cell membrane first occurs within the cell area facing the electrodes and (b) Different electroporation regimes: reversible $E_{\text{rev}} < E < E_{\text{irrev}}$ and irreversible $E > E_{\text{irrev}}$.

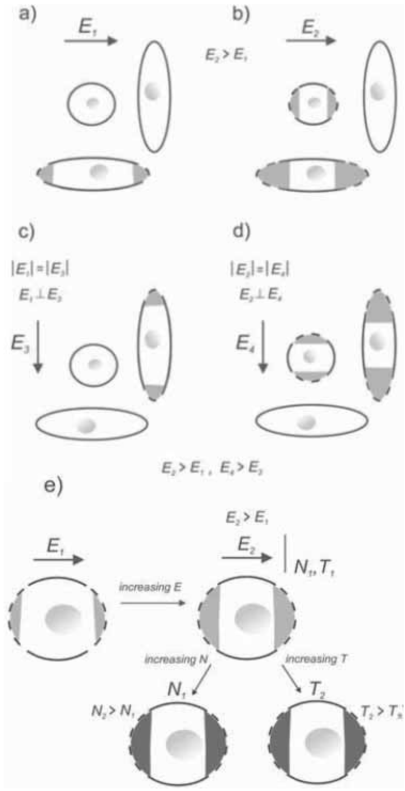

Figure 3. (a) Electric field parallel to elongated cell, (b) electric pulse amplitude is increased, (c) orientation of electric field is changed, (d) electric pulse amplitude is increased and (e) increasing the pulse amplitude results in larger area of membrane with smaller extent of electroporation, while increase in pulse number or duration does not affect the membrane area but increases the extent of electroporation.

environment enabling the authentication of users, statistical analysis, network traffic measurement, support for video streaming, as well as the use of various types of communications among users, such as forums, e-mail correspondence, videoconferencing [5].

2 The e-learning application

The main structure of the e-learning application is given in Figure 1.

The first part of our web-based e-learning application brings together the educational material on basic mechanisms underlying electroporation process on the levels of cell membrane, cell and tissues as a composite of cells. Electroporated cell in a local electric field exceeding reversible threshold value $E > E_{\text{rev}}$ is represented by a simple graphical illustration in Figure 2a: The electroporation of cell membrane first occurs within the cell area facing the electrodes (dashed line in Figure. 2a), since the induced transmembrane potential is maximal at the poles of the cell in accordance Schwan's equation: $U_{TI} = -1.5\, r\, E \cos\varphi$, where r is the radius of the cell, E is the strength of applied electric field, and φ is the angle between the direction of the electric field and the selected point on the cell surface. Different regimes of electroporation process, depending on parameters of the electric pulses applied, are illustrated in Figure 2b: the introduction of small molecules, macromolecules and cells' electrofusion require reversible electroporation regime ($E_{\text{rev}} < E < E_{\text{irrev}}$), while the permanent cell damaging requires irreversible electroporation thus local electric field exceeding irreversible threshold $E > E_{\text{irrev}}$.

The value of induced transmembrane voltage and thus the cell electroporation depends on the cell size, shape, and the position of the cell with respect to the direction of applied electric field, which we represented in Figures 3a,3b,3c and 3d. For a spheroidal cell, maximum induced transmembrane potential strongly depends on its orientation with the respect to the electric

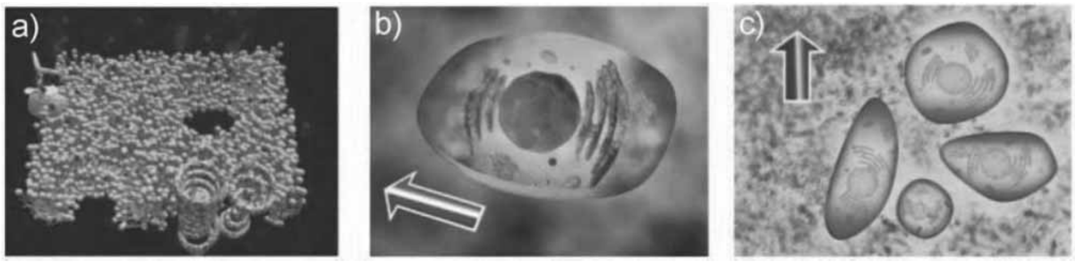

Figure 4. Introduction of small molecules (blue molecules) through a cell membrane (a) into an electroporated cell (b) and into the successfully electroporated cells within an exposed tissue (c)

field. It is maximum when the spheroidal cell is parallel to the applied electric field. In Figure 3e we illustrated that increasing the pulse amplitude results in larger area of membrane with smaller extent of electroporation, while increase in pulse number or duration does not affect the membrane area but increases the extent of electroporation. In order to visualize the electroporation process in three dimensions we used 3D Studio Max software. The animations of introduction of small molecules in 3D through an electroporated cell membrane, into an electroporated cell and into all successfully electroporated cells within an exposed tissue, which we included into the e-learning application, are shown in Figure 4.

The Modeling of local electric field distribution chapter provides an educational content about importance of visualization of local electric field distribution. The user is warned about possible errors that can be committed while performing cell or tissue electroporation, such as insufficient amplitude of electric pulses or inadequate electrode geometry, electrode positioning. This part of e-learning content is particularly intended as guidance to the practitioners who perform electrochemotherapy treatment of solid tumors. Namely, for successful tumor treatment all the clonogenic cells have to be destroyed, otherwise the tumor cell can regrow due to the insufficient magnitude of local electric field $E < E_{\mathrm{rev}}$. This was in our e-learning application demonstrated with an example of unsuccessful the skin tumor treatment performed on a nude mouse, as shown in Figure 5. The Figure 5a shows the electrode position and the tumor geometry just before the treatment, while Figure 5b shows the negative result of the treatment: two new tumors regrew in the regions (marked with 1 and 2 numbers) where the tumor tissue was not exposed to the sufficient electric field $E > E_{\mathrm{rev}}$.

By using simple graphical illustration we pointed out that the effectiveness of electrochemotherapy can be improved by: optimizing the applied voltage, changing electrode dimension or changing electrode orientation and their position, which we previously predicted by means of numerical modeling.

The chapter Important parameters of local electric field provides a list of important parameters of the local electric field distribution that affect the electrochemotherapy outcome, such as: electrode geometry (needle or plate electrodes), dimension of the particular electrode (width, length, diameter), distance between electrodes, electrode position with respect to the target tissue, electrode orientation with respect to the target tissue, geometry of the target tissue, geometry of the tissue surrounding the target tissue, the contact surface between the electrode and the tissue, electric properties of the target tissue i.e. tissue conductivity, electric properties of the surrounding tissue, the voltage applied to the electrodes and threshold values of the tissue E_{rev} and E_{irrev}. Using mathematical modeling and graphical illustrations we stressed the fact that the local electric field within the treated tissue is markedly non-homogeneous due to the specific structure and electric properties the tissues (particularly target tumor tissue that usually has higher

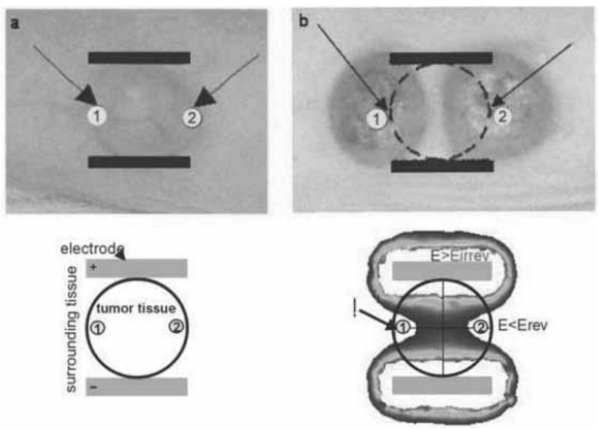

Figure 5. Unsuccessful tumor treatment performed on a nude mouse: a) The electrode position and the tumor geometry just before the treatment, b) after the treatment two new tumors regrew in the regions (marked with numbers 1 and 2) where the tumor tissue was not exposed to $E > E_{\mathrm{rev}}$.

electric conductivity than its surrounding tissues).

The chapter Local electric field distribution in 3D tissue models provides 2D and 3D animations of local electric field distribution in models of cutaneous and subcutaneous tumors, which we previously numerically calculated using COMSOL Multiphisycs software. We presented local electric field distribution in models of cutaneous protruding tumor and model of subcutaneous non-protruding tumor with skin.

The objective of this part of the e-learning application is to provide an interaction with the educational content in order to simulate the “hands-on” learning approach about the parameters of the local electric field distribution that have been previously explained. Namely, by varying different parameters (such as amplitude of electric pulses, electrodes’ dimensions and shape and distance between electrodes) in the navigation bar users have the possibility to shape the electric field distribution within the models (see the navigation bar in Figure 6). The local electric field distribution can be viewed in 2D model cross-sections or played as a 3D animation. The E is displayed in the range between E_{rev} to E_{irrev}.

In Figure 6 the local electric field distribution inside the cutaneous protruding tumor obtained with two different amplitudes of applied voltage (Figure 6a: $U = 300$ V and Figure 6b: $U = 600$ V) using two parallel plate electrodes is shown as example. By increasing the applied voltage (for the same tissue geometry, electrode size and position) the stronger local electric field is obtained. Similar effect can be achieved by increasing the electrode dimensions (electrode width) or changing electrode orientation, while by increasing the distance between electrodes the tumor is exposed to a lower local electric field intensity, as shown in Figure 6c and 6d.

The model of subcutaneous tumor gives the user an insight into the electric field distribution within the target tissue when electroporated through the skin. This model is composed of two layers; the upper layer representing skin tissue with lower specific conductivity compared to the underlying layer being more conductive. The electric field distribution is presented in two models with two different thicknesses of the skin layer: 1 mm (Figure 7a) and 3 mm (Figure 7b). Thus, the user can appreciate the influence of the skin thickness and its electric conductivity on the local electric field distribution within the target tumor and its surroundings. The key message is that in order to successfully electroporate the target tumor through the thicker skin layer (3 mm) a higher voltage need to be applied compared to

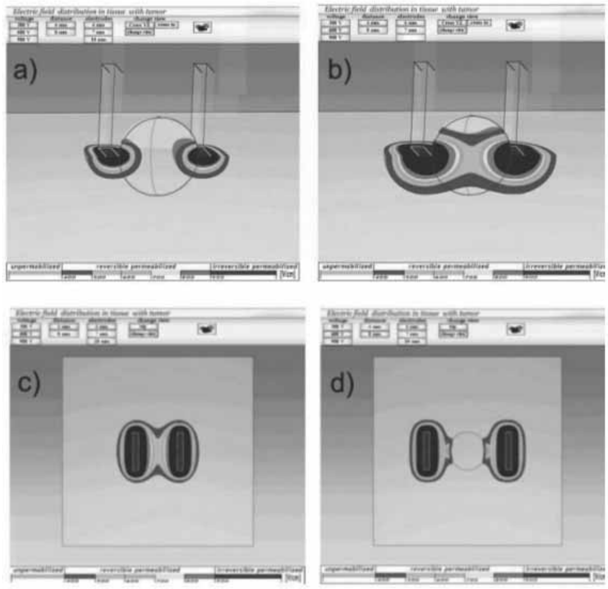

Figure 6. Electric field distribution inside the protruding tumor model for two different applied voltages on the electrodes: a) $U = 300$ V and b) $U = 600$ V. The electrodes are 4 mm wide and 4 mm apart in both cases. Electric field distribution inside the models for two distances between electrodes: c) $d = 4$ mm and d) $d = 8$ mm. The electrodes are 4 mm wide with the applied voltage $U = 600$ V in both cases.

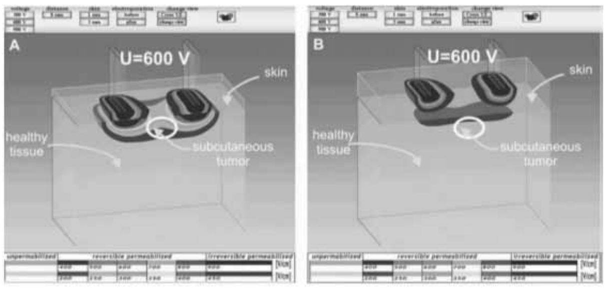

Figure 7. Electric field distribution within a model of subcutaneous tumors seeded below: a) a 1 mm thick skin layer; b) a 3 mm thick skin layer

the tumor electroporation through the thinner skin layer (1 mm). Based on this the user is offered a guideline on how to overcome the highly resistive skin tissue in order to permeabilize more conductive underlying tissues.

Based on the presented educational contend in chapter Local electric field distribution in 3D tissue models the user can appreciate that the plate electrodes are more suitable for treatment of protruding cutaneous tumors, while for situations when the tumor is more deeply seeded in the tissue the needle electrodes are to be used and that by increasing the number of needle electrodes

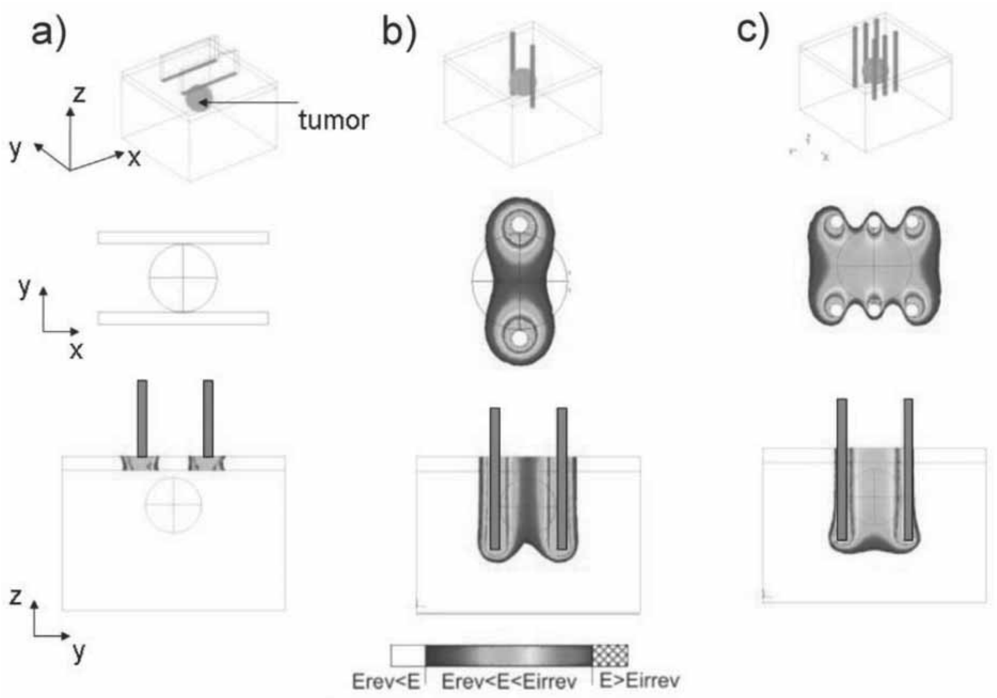

Figure 8. Local electric field distribution in subcutaneous tumor model using plate electrodes (a), a pair of needles (b) and three pairs of needles (c). The applied voltage in all cases is $U = 300$ V.

stronger local electric field in the tissue can be achieved, as shown in Figure 8.

3 Conclusions

We developed a web-based e-learning application that provides the educational content on important parameters that affect the effectives of cell and tissue electroporation. The e-learning application is particularly aimed at providing the knowledge about the adequate choice of local electric field parameters such as electric pulse amplitude, electrode shape and number or electrode positioning, being important in treatment planning of electrochemotherapy as well as in other electroporation based applications.

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